

The spatial resolution of the pinhole eyes of giant clams (*Tridacna maxima*)

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Giant clams (*Tridacna* spp.) have several hundred small pinhole-type eyes on the exposed mantle. They respond by withdrawing the mantle to movements of dark objects, even if these cast no shadow on the animal as a whole. I investigated this 'sight reaction' using black and white square-wave gratings whose phase abruptly changed so that the white areas became dark and vice versa. Gratings with periods of 13.5° were ineffective, but gratings of 20.7° caused partial retraction of mantles or siphons. This implies an acceptance angle for the best-resolving eyes of between 8.7° and 21.8° . A single black spot was effective if its angular diameter was 13.5° but not 11.7° . The mean threshold for the pure dimming of a large field was a decrease of 12.3%, but responses increased in strength up to a dimming of 35%. Anatomically the eyes are ca. 400 μm deep from aperture to receptors, the aperture has a mean diameter of 90 μm and the receptors are 25 μm across. This gives an angular acceptance angle for single receptors of 16.5° , which is completely consistent with the behavioural measurements.

Keywords: giant clam; *Tridacna*; mollusc; pinhole eye; resolution; vision

1. INTRODUCTION

Giant clams of various *Tridacna* species are common on tropical reefs. They have several hundred small (0.5 mm) dark eyespots around the border of the mantle, each of which contains a spherical or lozenge-shaped cavity with a small pupil-like aperture, and with a hundred or more photoreceptors in the base. The bulk of the cavity contains transparent cells, but these are not refractile, so that the structure acts optically as a simple pinhole eye (Stasek 1966; Wilkens 1986). Wilkens (1984) showed that the receptors all hyperpolarized to light and that they were of three spectral types, with maximum sensitivities in the blue-green (490 nm), blue (450 nm) and the ultraviolet (360 nm) areas of the spectrum. About half of the cells in Wilkens' sample responded to off-going light with a short train of action potentials and the other half did not. Similar hyperpolarizing, off-responding cells have been found in other bivalve molluscs: in scallops (*Pecten*; McReynolds & Gorman 1970) and file clams (*Lima*; Mpitsos 1973).

Giant clams respond to sudden dimming by withdrawing their siphons and mantles, and by partially closing their shells. This is presumably a defensive response to potential predators such as fishes, or to birds on an exposed reef. They do not respond in this way to increases in illumination, although Wilkens (1986) noticed that they do have an unrelated behaviour in which a change in the direction of the incident light results in a change in the orientation of the mantle. In addition to responding to dimming, giant clams will also respond to movements of objects, such as a hand, that cast no direct shadow. Wilkens (1986) refers to this as 'sight reaction'. Scallops show a similar reaction, closing their shells well before a moving object, such as a diver, has got close enough to cast a shadow (Land 1968). For such a response an image-forming optical system of some kind is essential, because the stimulus for the receptors is the local dimming of one part of the image relative to the rest, rather than dimming

of the eye as a whole. Although the response of the clams and scallops is usually to movement, the mechanism does not make use of true motion detectors of the kind found in insects. The response is instead caused by the sequential dimming of receptors across the image, caused by the movement of the image of a dark object. In general, the better the optical system linked to off-detectors the more effective it is in detecting predators at a distance. Other bivalves have come up with ingenious solutions to the problem of image formation: scallops use concave mirrors (Land 1965) and ark shells (*Arca* and *Pectunculus*) have numerous small compound eyes, resembling those of arthropods, around the mantle edge (Nilsson 1994). In giant clams the optical structures are pinhole eyes, in which the only mechanism for restricting the field of view of each receptor is the pigment that surrounds the aperture of the eye. The purpose of this paper is to determine how good this kind of eye can be at resolving spatial patterns, given that such eyes are not noted for their optical quality.

2. MATERIAL AND METHODS

Three giant clams (*Tridacna maxima*) were collected from the reef-top adjoining the Heron Island Marine Station of the University of Queensland. They were held in subdued daylight in aquaria with running sea water and were returned to the reef after the study was completed. The clams were of different colour morphs: clam 1 had a grey and silver mantle, clam 2 was dark blue and clam 3 was a russet brown. All three were between 15 cm and 16 cm in shell length. The observations were made between 10 and 13 June 2002 during daylight hours.

The principal stimuli used were high-contrast (80%) square-wave gratings with varying periods. The gratings were produced in POWERPOINT, and were presented to the clam on the screen of a laptop computer, placed screen-down above the aquarium containing the clam. The apparent distance of the nearest part of the clam's mantle to the screen (taking into account the pos-

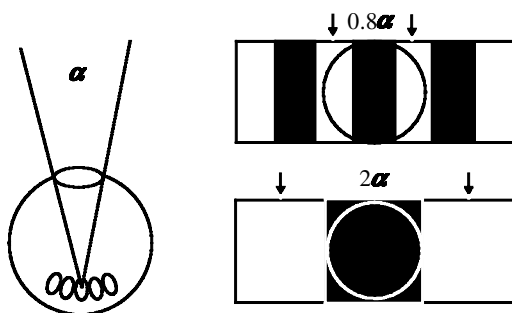


Figure 1. Method for determining the acceptance angle (α) of a receptor in an eye. When presented with a contrast-reversing grating of spatial period 0.8α , or narrower, there is no change in overall intensity within the acceptance angle, and no response should occur. For wider gratings dimming occurs when contrast reverses, and when the spatial period reaches 2α the receptor is fully darkened.

ition of air–water interface) was 13.2 cm, meaning that 1 cm on the screen subtended 4.35° when seen from the mantle. The total field occupied by the grating was *ca.* $80^\circ \times 60^\circ$ at the mantle. The luminance of the white regions of the screen was 100 cd m^{-1} .

The clams did not react to static stimuli, but responded very strongly to gratings whose phase changed suddenly, that is to say when all the white stripes changed to black and vice versa. This caused no net dimming, but presumably all those eyes that imaged a light stripe responded vigorously when it changed to dark (but not to a dark-to-light change). This response was used to determine the eyes' spatial resolution, as shown in figure 1. Supposing that the field of view of a receptor in an eye (i.e. the angle subtended by the pupil at that receptor) is α degrees, then a grating with a period of 2α should be an effective stimulus, because one stripe will occupy the whole field of view of at least some eyes. However, a grating with a period α should not be an effective stimulus because the field of view of a receptor will contain both a white and a dark stripe, and a receptor will see no net dimming when the stripes switch. (In fact this is only strictly true for square pupils, because circular pupils weight the centre more highly than the edges. For a circular pupil the cut-off will be close to 0.8α .) Thus, depending on the sensitivity of the cells to dimming, one would expect to see a change from no response to a definite response between spatial periods of 0.8α and 2α . This is indeed what was found (figure 3*a,b*).

The gratings were presented in a pseudo-random sequence, with two minutes between each change. Two minutes of a 50% grey screen was inserted between each grating pair. With this presentation rate, habituation of the response was minimal. Two other types of stimuli were also used and presented in the same way. These were single black spots of varying diameter, and dimming of the whole field ($80^\circ \times 60^\circ$) by varying amounts. As the greyscale of the computer was too coarse, weak greys were constructed from fine gratings of black lines, well below the clams' resolution threshold. Screen brightness was checked with an industrial photometer. Responses were recorded by direct observation. If the whole mantle retracted this was scored as 1, and if only part retracted this was scored 0.5. Clam 2 responded more weakly than the other two, but had a clearly visible response of the siphon, which withdrew slightly even to stimuli that had no effect on the mantle. For clam 2 either the siphon response or local retraction was recorded as 0.5. The siphon was not easily visible in clams 1 and 3.

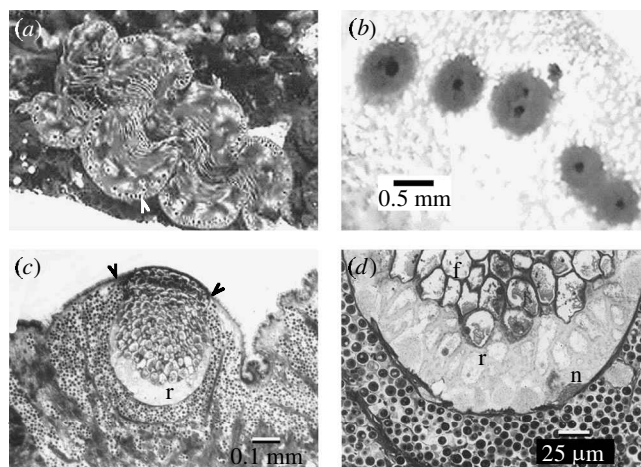


Figure 2. (a) Giant clam underwater showing the eyespots (arrowheads) surrounding the mantle. The length of the gape is 15 cm. (b) Five eyes from the mantle of clam 1, showing the apertures, which are much smaller than in histological preparations (c). Note the unusual double aperture in the middle eye. (c) Section through a single eye showing the positions of the receptors (r) and the limits of the pigmented aperture (arrowheads). (d) Higher-power magnification section of the receptor region: f, 'filler' cells; r, receptors; n, nerve bundle.

A 1 cm strip of mantle containing about 10 eyes was removed from clam 1 and fixed (4% paraformaldehyde, 0.25% glutaraldehyde and 2% sucrose in phosphate buffer), dehydrated and embedded in resin. It was sectioned at $2 \mu\text{m}$ and stained with toluidine blue. The clam appeared to have recovered and was normally responsive 24 h later.

3. RESULTS

(a) *Anatomy of the eye: field of view of the receptors*

The sizes of the eyes and pupils of five eyes from the central mantle of clam 1 were measured under the microscope in the live animal (figure 2*b*). Eye diameter ranged from $400 \mu\text{m}$ to $600 \mu\text{m}$ (mean $500 \mu\text{m}$), and pupils from $85 \mu\text{m}$ to $107 \mu\text{m}$ (mean $90 \mu\text{m}$). The other two clams gave similar values. From the section shown in Wilkens (1986, fig. 3*A*) and our own (figure 2*c,d*), it appears that the receptors are situated at a distance of *ca.* $400 \mu\text{m}$ from the pupil in an eye with an external diameter of 0.5 mm . At this distance, a $90 \mu\text{m}$ pupil subtends an angle of 12.9° at the receptor. This angle should correspond to the receptor's field of view (α ; figure 1), provided that the receptors are small. However, the receptors have a diameter of *ca.* $25 \mu\text{m}$ (see figure 2*d*) that broadens this angle by $25/400$ radians, or 3.6° , so that the total acceptance angle of a receptor becomes 16.5° .

The histological appearance of an eye is shown in figure 2*c,d*. The chamber of the eye itself is ovoid, $390 \mu\text{m}$ deep and $280 \mu\text{m}$ wide (uncorrected values). The distal two-thirds of the chamber contains cells with well-defined walls whose contents (possibly mucus) have shrunk in the fixative ('f' in figure 2*d*). In the aperture itself, the cells are flattened and stain more strongly and there is a definite cornea-like layer of connective tissue covering the surface. Beneath these 'filler' cells lie the receptors, and these line the bottom and much of the sides of the chamber. This

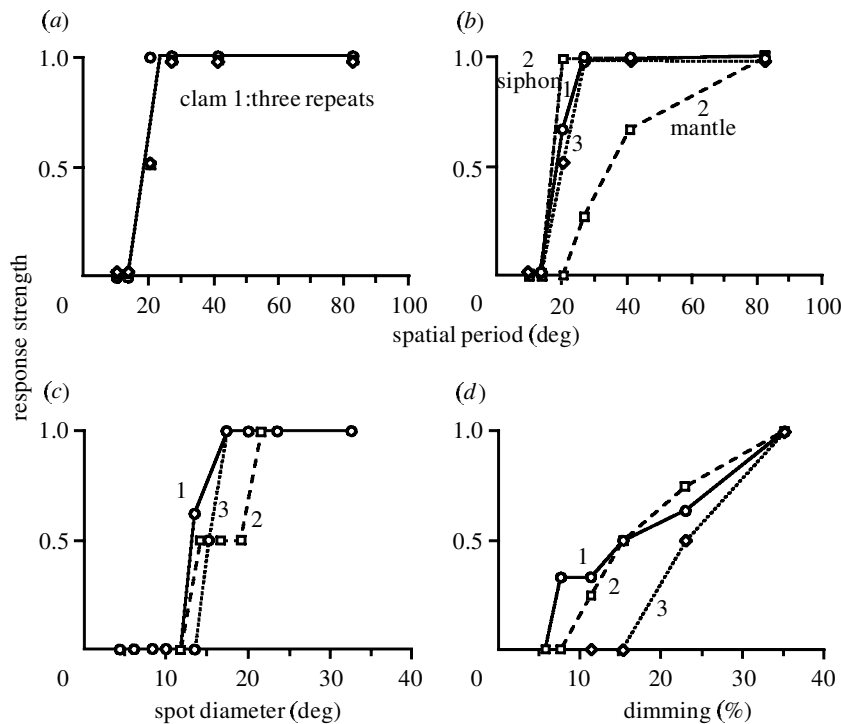


Figure 3. (a) Responses of clam 1 to phase reversals of gratings with increasing spatial periods. In all three repeats the threshold lies between 13.9° and 20.7° . (b) As (a), but showing the mean results for three clams. Clam 2 had a higher threshold for the mantle response, but the siphon withdrawal had the same threshold as the mantle responses of clams 1 and 3. 1, Solid line; 2 siphon, dashed line; 2 mantle, dot-dashed line; 3, dotted line. (c) Mean responses of the three clams to the appearance of black spots of different diameters. The thresholds are in the range of 11.7 – 15.2° . 1, Solid line; 2, dashed line; 3, dotted line. (d) Responses to dimming. The thresholds vary from 7 to 20%, and the responses show more gradual summation than with the other stimulus types. 1, Solid line; 2, dashed line; 3, dotted line.

means that the field of view of the eye as a whole is at least 90° . There are two receptor layers across the bottom, decreasing to one layer up the sides. The receptors each have a nucleus and weakly staining cytoplasm surrounded by a rather angular membrane that appears rough at higher powers. This is probably where the photopigment is situated. The receptors are *ca.* $25\ \mu\text{m}$ in diameter, and are embedded in a deeper staining matrix. Some receptors have nerve fibres leaving them and several small nerve bundles could be seen beneath the receptor layer (n, figure 2d). A rough estimate indicates that there are *ca.* 250 receptors in an eye of typical size. This description accords well with the anatomical studies of Wilkens (1986) and Stasek (1966).

The aperture of the eye in the sections was much larger than in life (arrowheads in figure 2c; compare with figure 2b). In this section the aperture was $250\ \mu\text{m}$ wide as opposed to $90\ \mu\text{m}$ in the living animal. This appears to be the effect of the fixative, rather than dehydration, as three other fixed but otherwise untreated eyes had aperture diameters in the range 206 – $241\ \mu\text{m}$. This increase in diameter is also apparent in the drawing given by Wilkens (1986). Thus, histology on its own gives a quite misleading picture of the eye's potential resolution, since a receptor looking out through a $250\ \mu\text{m}$ aperture will have a field of view of 36° , more than twice as large as it would in life.

(b) Spatial resolution thresholds

The responses of clam 1 to phase switches of a series of gratings are shown in figure 3a. The responses are extremely consistent between repeats. They show that

there are no responses to gratings with a 10.3° or 13.9° period, partial responses to 20.7° gratings and complete responses to 27.4° gratings. Clearly, some receptors are responding to gratings with 20.7° periods, so the threshold lies between this and 13.9° . If the acceptance angle of a receptor (α , figure 1) is 16.5° , then one would expect a threshold of between 0.8 and 2 times this value (see figure 1), i.e. between 13.2° and 33° . Thus, the range measured behaviourally is within the expected range. Alternatively, if the grating threshold is taken to be half way between 13.9° and 20.7° (i.e. 17.4°) the corresponding values of α lie between $17.4/2^\circ$ and $17.4/0.8^\circ$, i.e. between 8.7° and 21.8° , which again is consistent with the anatomically estimated value (16.5°).

Figure 3b shows the average responses of all three clams. Clams 1 and 3 gave essentially identical responses, but clam 2 was different. For the mantle response the threshold was higher ($> 20.7^\circ$) and the clam did not make consistent complete mantle responses until the grating period reached 80° (i.e. one period occupied the whole of the screen). However, the response of the siphon was very different, and much more like the responses of the other two clams. Again, there was no response to the 13.9° period gratings, but a definite response to the 20.7° gratings, and this was true for all three repeats. It seems that the fundamental receptor threshold for clam 2 was the same as clams 1 and 3, but that it needed much more summation of individual receptor responses to produce a full mantle retraction. Clam 2 also showed response gradation over a much wider range of spatial periods than the other two clams.

(c) Response to single spots

Single black spots presented on a light background also produced mantle and siphon responses (figure 3c). Spots subtending 11.7° or less produced no responses, but spots of 13.5° produced weak responses in clams 1 and 2, as did spots of 15.2° in clam 3. All produced full mantle retraction to 20° spots. These responses are clearly to the images of the dots rather than to the overall dimming that they cause, because a 13.5° dot only causes an overall dimming of 3% in an $80^\circ \times 60^\circ$ field, and this is well below the 12.3% threshold for wide field dimming that is reported in § 3d.

The threshold of between 11.7° and 13.5° is somewhat lower than the estimated receptor acceptance angle of 16.5° obtained from eye anatomy, and it implies that at threshold no cells are fully darkened. The maximum amount of dimming of any one receptor would be *ca.* 60%; well above the overall dimming threshold of 12.3%.

(d) Responses to dimming

The responses of the three clams to dimming of the whole $80^\circ \times 60^\circ$ field are shown in figure 3d. Thresholds varied between *ca.* 7% (clam 1) and 20% (clam 3). The mean was 12.3%. In all three, full mantle retraction was not seen until dimming reached 35%. The responses to dimming show much more gradation than to the gratings or the single spots. This is presumably because, with both gratings and spots, the stimulus strength increases very rapidly once the threshold is reached: for spots, the amount of dimming increases as the square of the spot diameter, whereas with dimming the increase is linear.

4. DISCUSSION

I have shown here that the pinhole eyes of giant clams produce images that can resolve stripe patterns with periods of between 13.9° and 20.7° . These figures are quite consistent with pinhole optics that provide each receptor with a field of view *ca.* 16.5° in angular diameter. It is also shown that *Tridacna* can respond to dark targets subtending at least 13.5° . This would correspond to something like a 50 cm-wide diver at 2.1 m, or a 10 cm-diameter fish at 42 cm. These figures are not spectacular (scallops, with a better optical system, can see objects 2° across (Land 1966)), but they afford a measure of protection that an animal with only a shadow response lacks. A shadow will not be cast until the potential predator is literally on top of the mollusc prey, whereas the giant clam has almost 1 s to respond to a fish first seen at 42 cm and swimming at 0.5 m s^{-1} . This should allow enough time for the mantle to be retracted out of harm's way.

Pinhole or pigment-pit eyes are common throughout the lower phyla, where they are generally used to orient the animal towards or away from the general direction of light (Fraenkel & Gunn 1961; Land & Nilsson, 2002). For these purposes the images in the eyes can be very crude and the aperture, whose diameter determines image quality, can be correspondingly wide. There are only two documented cases of pinhole eyes that produce images with more sophisticated functions than this: the giant clams discussed here, and *Nautilus*, the relict cephalopod famous for its beautiful shell. *Nautilus* was studied by

Muntz & Raj (1984), who examined its optomotor response by placing an animal in a dish surrounded by a rotating vertical-stripe pattern. Provided that the animal could resolve the stripes, it swam around in the dish following the rotation of the stripes. (In the real-world environment, which does not itself rotate, the function of this response is to hold the animal's rotational velocity at zero, i.e. to prevent involuntary yaw). Muntz & Raj (1984) found that the response ceased when the spatial period of the grating fell below 11° . With a 1 mm pupil in an eye of 10 mm in diameter, each receptor should view a 5.7° -wide field. Using the argument presented here one would expect a cut-off between 5.7° and 11.4° . As in the case of *Tridacna*, this expectation seems to be fulfilled. Thus, in the two cases for which information is available, it seems that pinhole eyes perform as well as can be expected from their minimal optical systems. It could be argued that both kinds of eye would benefit from the inclusion of a lens, and in terms of both resolution and sensitivity that is undoubtedly true. However, if *Tridacna* responded to everything in its visual world it would spend most of its life shut, and it is possible that its poor optics provide just the kind of filter that prevents this. It is harder to make that case for *Nautilus*, however, which has a much larger eye, and whose relatives, the octopuses and squids, have excellent vision that rivals that of most vertebrates.

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